AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (Original) A nitrile hydratase comprising an α-subunit and a β-subunit, wherein the α-subunit has an amino acid sequence in which at least one amino acid of the 36th, 71st, 148th and 204th amino acids in the amino acid sequence of SEQ ID NO: 1 in the Sequence Listing is substituted by another amino acid.

2. (Original) The nitrile hydratase according to claim 1, wherein at least one amino acid of the 6th, 19th, 38th, 77th, 90th, 102nd, 106th, 126th, 130th, 142nd, 146th, 187th, 194th and 203rd amino acids in the amino acid sequence of the α-subunit is further substituted by another amino acid.

3. (Original) The nitrile hydratase according to claim 1 or 2, wherein the β-subunit has the amino acid sequence of SEQ ID NO: 2 in the Sequence Listing.

4. (Original) The nitrile hydratase according to claim 3, wherein at least one amino acid of the 10th, 32nd, 37th, 41st, 46th, 48th, 51st, 72nd, 118th, 127th, 146th, 160th, 186th and 217th amino acids in the amino acid sequence of the β-subunit is further substituted by another amino acid.

5. (Original) The nitrile hydratase according to claim 4, wherein at least one amino acid of the 20th, 21st, 108th, 200th and 212th amino acids in the amino acid sequence of the β-subunit is further substituted by another amino acid.

6. (Currently Amended) The nitrile hydratase according to ~~any one of claims 1 to 5~~ claim 1, wherein at sites other than the amino acid substitution sites in the amino acid sequence carried by at least one of the β-subunit and the α-subunit, one or several amino acids are subject to substitution, insertion or deletion within the scope of not impairing the nitrile hydratase activity.

7. (Original) A nitrile hydratase comprising an α-subunit and a β-subunit, wherein the β-subunit has an amino acid sequence in which at least one amino acid of the 10th, 32nd, 37th, 41st, 46th, 48th, 51st, 72nd, 118th, 127th, 146th, 160th, 186th and 217th amino acids in the amino acid sequence of SEQ ID NO: 2 in the Sequence Listing is substituted by another amino acid.

8. (Original) The nitrile hydratase according to claim 7, wherein at least one amino acid of the 20th, 21st, 108th, 200th and 212th amino acids in the amino acid sequence of the β-subunit is further substituted by another amino acid.

9. (Original) The nitrile hydratase according to claim 7 or 8, wherein the α-subunit has the amino acid sequence of SEQ ID NO: 1 in the Sequence Listing.

10. - 22 (Canceled)

23. (Original) A gene encoding the nitrile hydratase, comprising the gene which codes for the amino acid sequence of the α-subunit and the gene which codes for the amino acid sequence of the β-subunit of nitrile hydratase, wherein the amino acid sequence of the α-subunit is an amino acid sequence in which at least one amino acid of the 36th, 71st, 148th and 204th amino acids in the amino acid sequence of SEQ ID NO: 1 in the Sequence Listing is substituted by another amino acid.

24. (Original) The gene according to claim 23, wherein at least one amino acid of the 6th, 19th, 38th, 77th, 90th, 102nd, 106th, 126th, 130th, 142nd, 146th, 187th, 194th and 203rd amino acids in the amino acid sequence of the α-subunit is further substituted by another amino acid.

25. (Original) The gene according to claim 23 or 24, wherein at sites other than the amino acid substitution sites carried by the amino acid sequence of the α-subunit, one or several amino acids are subject to substitution, insertion or deletion within the scope of not impairing the nitrile hydratase activity.

26. (Original) The gene according to claim 23, wherein the gene encoding the amino acid sequence of the α-subunit has a base sequence obtained by substituting at least one base sequence among the 106th to 108th, 211th to 213th, 442nd to 444th, and 610th to 612th of the base sequence of SEQ ID NO: 3 in the Sequence Listing with another base sequence.

27. (Original) The gene according to claim 26, wherein at least one base sequence among the 16th to 18th, 55th to 57th, 112th to 114th, 229th to 231st, 268th to 270th, 304th to 306th, 316th to 318th, 376th to 378th, 388th 390th, 424th to 426th, 436th to 438th, 559th to 561st, 580th to 582nd, and 607th to 609th of the base sequence is further substituted by another base sequence.

28. - 34. (Canceled)

35. (Original) A gene encoding the nitrile hydratase, comprising the gene which codes for the amino acid sequence of the α-subunit and the gene which codes for the amino acid sequence of the β-subunit of nitrile hydratase, wherein the amino acid sequence of the β-subunit is an amino acid sequence in which at least one amino acid of the 10th, 32nd, 37th, 41st, 46th, 48th, 51st, 72nd, 118th, 127th, 146th, 160th, 186th and 217th amino acids in the amino acid sequence of SEQ ID NO: 2 in the Sequence Listing is substituted by another amino acid.

36. (Original) The gene according to claim 35, wherein at least one amino acid of the 20th, 21st, 108th, 200th and 212th amino acids in the amino acid sequence is further substituted by another amino acid.

37. (Original) The gene according to claim 35 or 36, wherein at sites other than the amino acid substitution sites carried by the amino acid sequence of the β-subunit, one or several amino acids are subject to substitution, insertion or deletion within the scope of not impairing the nitrile hydratase activity.

38. (Original) The gene according to claim 35, wherein the base sequence encoding the amino acid sequence of the β-subunit has a base sequence obtained by substituting at least one base sequence among the 28th to 30th, 94th to 96th, 109th to 111th, 121st to 123rd, 136th to 138th, 142nd to 144th, 151st to 153rd, 214th to 216th, 352nd to 354th, 379th to 381st, 436th to 438th, 478th to 480th, 556th to 558th, and 649th to 651st of the base sequence as set forth in SEQ ID NO: 4 in the Sequence Listing with another base sequence.

39. (Original) The gene according to claim 38, wherein at least one base sequence among the 58th to 60th, 61st to 63rd, 322nd to 324th, 598th to 600th, and 634th to 636th of the base sequence is further substituted by another base sequence.

40. - 49 (Canceled)

50. (Currently Amended) A plasmid having the gene encoding the nitrile hydratase according to any one of claims 23, 24, 26, 27, 35, 36, 38 and 39 [[to 46]].

51. (Currently Amended) The plasmid according to ~~any one of claims 47 to~~ claim 50, which comprises the constitution for the expression of the nitrile hydratase in a host cell.

52. (Original) A transformant obtained by transforming a host cell with the plasmid according to claim 51.

53. (Original) A method for production of nitrile hydratase, comprising a step of cultivating the transformant according to claim 52 in a culture medium and producing a nitrile hydratase based on the nitrile hydratase gene carried by the plasmid in the transformant.

54. (Original) The method for production according to claim 53, further comprising a step of recovering nitrile hydratase from the transformant after the cultivation, the culture and a product of processing them.

55. (Currently Amended) A method for production of nitrile compound by bringing a nitrile compound into contact with a nitrile hydratase in an aqueous medium to obtain a corresponding nitrile compound, wherein the nitrile hydratase is one according to any one of claims 1, 2 and 4 to 8 [[to 12]].

56. (Original) A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity, V_{max}, K_m, thermal stability, stability against the substrate and stability against the product, by specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

(a) aligning the amino acid sequence of the enzyme having the nitrile hydratase activity before modification, with the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing and the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing,

(b) specifying, based on the results of the alignment, the amino acid residues corresponding to the region extending from the 36th threonine to the 48th asparagine in the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing, and to the region extending from the 31st lysine to the 51st phenylalanine and to the region extending from the 112th lysine to the 127th leucine in the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing, and

(c) performing substitution, insertion or deletion at one or more sites of the specified amino acid residues.

57. (Original) A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity, V_{max}, K_m, thermal stability, stability against the substrate and stability against the product, by specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

(d) aligning the amino acid sequence of the enzyme having the nitrile hydratase activity before modification, with the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing and the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing,

(e) specifying, based on the results of the alignment, the amino acid residues corresponding to the 36th, 48th, 71st, 148th, 188th and 204th of the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing, and of the amino acid residues corresponding to the 10th, 32nd, 33rd, 37th, 40th, 41st, 46th, 48th, 51st, 61st, 72nd, 112th, 118th, 127th, 146th, 150th, 160th, 168th, 171st, 176th, 186th, 217th and

218th of the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing, and

(f) performing substitution, insertion or deletion at one or more sites of the specified amino acid residues.

58. (Original) A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity, V_{max}, K_m, thermal stability, stability against the substrate and stability against the product, by specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

(g) inferring the stereostructure of the enzyme having the nitrile hydratase activity before modification by carrying out an alignment based on the nitrile hydratase stereostructure and the amino acid sequence as set forth in PDB (Protein Data Bank) ID NO: 1IRE,

(h) specifying, based on the stereostructure inferred, the amino acid residues in the regions corresponding to the 2nd helix as counted from the N-terminal in Chain 1IRE: A, and to the 1st helix and the 2nd helix from the N-terminal, the loop portions inserted in the latter helices and the 3rd helix as counted from the C-terminal in Chain 1IRE: B in the nitrile hydratase stereostructure as set forth in PDB ID NO: 1IRE, and

(i) performing substitution, insertion or deletion at one or more sites of the specified amino acid residues.

59. (Original) A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the

enzymatic activity, substrate specificity, V_{max}, K_m, thermal stability, stability against the substrate and stability against the product, by specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

(j) inferring the stereostructure of the enzyme having the nitrile hydratase activity before modification by carrying out an alignment based on the nitrile hydratase stereostructure and the amino acid sequence as set forth in PDB ID NO: 1IRE,

(k) specifying, based on the stereostructure inferred, the four amino acid residues such as the amino acid residues which correspond to the 89th amino acid residue glutamine and the 165th amino acid residue glutamic acid as counted from the N-terminal in Chain A, and the amino acid residues which correspond to the 37th amino acid residue phenylalanine and the 48th amino acid leucine as counted from the N-terminal in Chain B in the nitrile hydratase stereostructure as set forth in PDB ID NO: 1IRE,

(l) specifying the amino acid residues whose side-chain front-end heavy atoms are located within 5Å of radius in the respective stereostructures having each of the side-chain front-end heavy atoms of the four above-specified amino acid residues as the point center, and

(m) performing substitution, insertion or deletion at one or more of the amino acid residues specified in the above (l).

60. (Original) A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity, V_{max}, K_m, thermal stability, stability against

the substrate and stability against the product, by specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

(n) inferring the stereostructure of the enzyme having the nitrile hydratase activity before modification by carrying out an alignment based on the nitrile hydratase stereostructure and the amino acid sequence as set forth in PDB ID NO: 1IRE,

(o) specifying, based on the inferred stereostructure, the region which forms a cavity through which a substrate passes from the outside of the enzyme toward the active center, or a product passes from the active center to the outside of the enzyme,

(p) specifying, among the amino acid residues constituting the above-specified region, the amino acid residues whose alteration leads to a change in the cavity size and further controls the easiness or difficulty in passing of the substrate/product, and

(q) performing substitution, insertion or deletion at one or more of the amino acid residues specified in the above (p).

61. (Original) A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity, V_{max}, K_m, thermal stability, stability against the substrate and stability against the product, by specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

(r) inferring the stereostructure of the enzyme having the nitrile hydratase activity before modification by carrying out an alignment based on the nitrile hydratase stereostructure and the amino acid sequence as set forth in PDB ID NO: 1IRE,

(s) specifying, based on the stereostructure inferred, the four amino acid residues such as the amino acid residues which correspond to the 89th amino acid glutamine (A89Q) and the 165th amino acid glutamic acid (A165E) as counted from the N-terminal in Chain A, and the amino acid residues which correspond to the 37th amino acid phenylalanine (B37F) and the 48th amino acid leucine (B48L) as counted from the N-terminal in Chain B in the nitrile hydratase stereostructure as set forth in PDB ID NO: 1IRE,

(t) specifying the amino acid residues which effect a change in at least one of d1 to d3, when the shortest distance between the heavy atoms of the amino acid residue corresponding to A165E and of the amino acid residue corresponding to B48L is designated as d1; the shortest distance between the heavy atoms of the amino acid residue corresponding to A89Q and of the amino acid residue corresponding to B48L as d2; and the shortest distance between the heavy atoms of the amino acid residue corresponding to B37F and of the amino acid residue corresponding to B48L as d3, and

(u) performing substitution, insertion or deletion at one or more sites of the specified amino acid residues.

62. (Original) The method for modification according to claim 61, wherein the step of (t) is replaced by the following step (t'):

(t') specifying the amino acid residues which effect a change in at least one of d1 to d5, when the shortest distance between the heavy atoms of the amino acid residue corresponding to A165E and of the amino acid residue corresponding to B48L is designated as d1; the shortest distance between the heavy atoms of the amino acid residue corresponding to A89Q and of the amino acid residue corresponding to B48L as d2; the shortest distance between the heavy atoms of the amino acid residue corresponding to B37F and of the amino acid residue corresponding to B48L is designated as d3; the shortest distance between the heavy atoms of the amino acid residue corresponding to A165E and of the amino acid residue corresponding to B37F as d4; and the shortest distance between the heavy atoms of the amino acid residue corresponding to A89Q and of the amino acid residue corresponding to B37F as d5.

63. (Original) The method for modification according to any one of claims 56 to 62, wherein the enzyme having the nitrile hydratase activity before modification comprises the two polypeptides [A] and [B] of the following:

[A] a polypeptide consisting of an amino acid sequence which shows homology of at least 40% with the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing, and

[B] a polypeptide consisting of an amino acid sequence which shows homology of at least 25% with the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing.

64. (Original) The method for modification according to claim 63, characterized in that the polypeptide of [A] is the polypeptide of the following [C], and the polypeptide of [B] is the polypeptide of the following [D]:

[C] a polypeptide consisting of any amino acid sequence selected from the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing; the amino acid sequence in which substitution, insertion or deletion has been implemented at one or more sites in the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing; and the amino acid sequence in which at least one amino acid of the 6th, 19th, 38th, 77th, 90th, 102nd, 106th, 126th, 130th, 142nd, 146th, 187th, 194th and 203rd amino acids in the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing is substituted by another amino acid, and

[D] a polypeptide consisting of any amino acid sequence selected from the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing; the amino acid sequence in which substitution, insertion or deletion has been implemented at one or more sites in the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing; and the amino acid sequence in which at least one amino acid of the 20th, 21st, 108th, 200th and 212th amino acids in the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing is substituted by another amino acid.

65. (Original) The method for modification according to claim 63, wherein the polypeptide of [A] is the polypeptide of the following [E], and the polypeptide of [B] is the polypeptide of the following [F]:

[E] a polypeptide consisting of an amino acid sequence showing homology with the amino acid sequence which is encoded by the open reading frame (ORF)

composed of from the 704th to 1315th of the base sequence as set forth in SEQ ID NO: 104 in the Sequence Listing, and

[F] a polypeptide consisting of an amino acid sequence showing homology with the amino acid sequence which is encoded by the ORF composed of from the 1st to 680th of the base sequence as set forth in SEQ ID NO: 104 in the Sequence Listing.

66. (Original) A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity, V_{max}, K_m, thermal stability, stability against the substrate and stability against the product, by specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

(d') aligning the amino acid sequence of the enzyme having the nitrile hydratase activity before modification, with the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing,

(e') specifying, based on the results of the alignment, the amino acid residues corresponding to the 48th and 51st in the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing, and

(f') performing substitution, insertion or deletion at one or more sites of the specified amino acid residues, wherein among the two polypeptides constituting the enzyme having the nitrile hydratase activity before modification, one is the polypeptide of [E] according to claim 65 and the other is the polypeptide of [F] according to claim 65.

67. (Original) The method for modification according to claim 63, wherein the polypeptide of [A] is the polypeptide of the following [G]:

[G] a polypeptide containing the region as represented by the amino acid sequence $X_1CXLC_1SC_2X_2X_3X_4X_5$ (wherein C corresponds to cysteine, X to serine or threonine, L to leucine, C_1 to cysteine sulfinic acid (cysteine sulfinic acid·3-sulfinoalanine), S to serine, and C_2 to cysteine sulfenic acid (cysteine sulfenic acid·S-hydroxy-cysteine); and X_1, X_2, X_3, X_4 and X_5 represent arbitrary amino acid, respectively).

68. (Original) The method for modification according to claim 67, wherein X_1 is valine, X_4 is tryptophan, and X_5 is praline.

69. (Original) The method for modification according to claim 68, wherein X_2 is tyrosine and X_3 is praline.

70. (Currently Amended) The method for modification according to ~~any one of claims 67 to 69~~ claim 67, wherein bonding with a metal atom is located in the region represented by $X_1CXLC_1SC_2X_2X_3X_4X_5$.

71. (Original) The method for modification according to claim 70, wherein the metal atom is a cobalt atom.

72. (Currently Amended) A modified enzyme obtained by the method for modification according to claim 63 ~~any one of claims 56 to 71~~.

73. (Original) The gene encoding the modified enzyme according to claim 72.

74. (Original) A plasmid containing the gene according to claim 73.

75. (Currently Amended) A transformant obtained by transformation of a microorganism with the gene according to claim 73 ~~or the plasmid according to claim 74~~.

76. (Original) A method for production of a modified enzyme, comprising the step of recovering a modified enzyme from a culture obtained from cultivating the transformant according to claim 75, the cultivated cells or a product obtained from the processing of the culture or the cultivated cells.

77. (Currently Amended) A method for production of an amide compound, comprising the step of bringing the modified enzyme that is obtained from a culture obtained from cultivating the transformant according to claim 75, the cultivated cells or a product obtained from the processing of the culture or the cultivated cells, ~~or the method for production according to claim 76,~~ into contact with a nitrile compound in a solvent to convert the nitrile compound to a corresponding amide compound.

78. (New) A modified enzyme obtained by the method for modification according to claim 64.

79. (New) A modified enzyme obtained by the method for modification according to claim 65.

80. (New) A modified enzyme obtained by the method for modification according to claim 66.

81. (New) A modified enzyme obtained by the method for modification according to claim 67.